ADVANCED PROTEOMIC CHARACTERIZATION OF THE 26S PROTEASOME IN ARABIDOPSIS REVEALS INSIGHTS INTO COMPOSITION AND ASSEMBLY

by

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The dissertation is approved by the following members of the Final Oral Committee:
Richard D. Vierstra, Professor, Genetics
Richard Amasino, Professor, Genetics
Josh Coon, Professor, Chemistry
Donna Fernandez, Professor, Botany
Patrick Masson, Professor, Genetics

To Erin, my wife.

ACKNOWLEDGMENTS

Science doesn't purvey absolute truth. Science is a mechanism, a way of trying to improve your knowledge of nature. It's a system for testing your thoughts against the universe and seeing whether they match. This works not just for the ordinary aspects of science, but for all of life.

— Isaac Asimov (1988)

Acknowledgements go here.

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LIST OF ABBREVIATIONS AND ACRONYMS

API	Application programming interface
BCA	Bicinchoninic acid protein assay
BLAST	Basic local alignment search tool
C#	C sharp, a programming language
Da	Dalton, the atomic mass unit
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ESI	Electrospray ionization
E-value	Expectation value
FASTA	A format for storing protein sequences
FDR	False discovery rate
GUI	Graphical user interface
HCD	Higher-energy collisional dissociation
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
m	Mass
min	Minute
MS	Mass spectrometry

MS^1	Survey mass analysis
MS/MS	Tandem mass spectrometry
NCE	Normalized collision energy
nLC	Nanoflow liquid chromatography
ppm	Part per million
PSM	Peptide-spectrum match
PTM	Post-translational modification
s	Second
SILAC	Stable isotope labeling by amino acids in cell culture
S/N	Signal-to-noise ratio
TMT	Tandem mass tag

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David C. Gemperline

Under the supervision of Professor Richard D. Vierstra

At the University of Wisconsin-Madison

FIXME: basically a placeholder; do not believe

I did some research, read a bunch of papers, published a couple myself, (pick one):

1. ran some experiments and made some graphs,
2. proved some theorems

and now I have a job. I've assembled this document in the last couple of months so you will let me leave. Thanks!

Richard D. Vierstra

ABSTRACT

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Chapter 1

THE UBIQUITIN 26S PROTEASOME SYSTEM

1.1. Ubiquitin Conjugating Machinery

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1.3. Proteasome Expression

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1.4. Proteasome Assembly

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1.5. Proteasome Post-Translational Modification

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1.7. Proteasome Interacting Proteins

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Chapter 2

MORPHEUS SPECTRAL COUNTER: A COMPUTATIONAL TOOL FOR LABEL-FREE QUANTITATIVE MASS SPECTROMETRY USING THE MORPHEUS SEARCH ENGINE

2.1. Summary

Label-free quantitative MS based on the Normalized Spectral Abundance Factor (NSAF) has emerged as a straightforward and robust method to determine the relative abundance of individual proteins within complex mixtures. Here, we present Morpheus Spectral Counter (MSpC) as the first computational tool that directly calculates NSAF values from output obtained from Morpheus, a fast, open-source, peptide-MS/MS matching engine compatible with high-resolution accurate-mass instruments. NSAF has distinct advantages over other MS-based quantification methods, including a higher dynamic range as compared to isobaric tags, no requirement to align and re-extract MS1 peaks, and increased speed. MSpC features an easy to use graphic user interface that additionally calculates both distributed and unique NSAF values to permit analyses of both protein families and isoforms/proteoforms. MSpC determinations of protein concentration were linear over

several orders of magnitude based on the analysis of several high-mass accuracy datasets either obtained from PRIDE or generated with total cell extracts spiked with purified Arabidopsis 20S proteasomes. The MSpC software was developed in C# and is open sourced under a permissive license with the code made available at `http://dcgemperline.github.io/Morpheus_SpC/`.

2.2. Introduction

2.3. Methods

2.4. Results and Discussion

2.5. Conclusions

2.6. References

COLOPHON

This document was typeset with LaTeX using a MiKTeX distribution. It is based on the University of Wisconsin dissertation template created by William C. Benton (available at `https://github.com/willb/wi-thesis-template`).